Behavioural, population, and genetic processes affecting metapopulation dynamics of the Glanville fritillary butterfly

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Academic dissertation

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I Sarhan, A. 2006. Isolation and characterization of five microsatellite loci in the Glanville fritillary butterfly (*Melitaea cinxia*). Mol. Ecol.Notes 6:163-164.

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III Sarhan, A. and Haikola, S. Inbreeding depression following establishment of new populations in a natural metapopulation of the Glanville fritillary butterfly (*Melitaea cinxia*) (submitted)

IV Sarhan, A. Protandry in the Glanville fritillary butterfly (*Melitaea cinxia*): early emergence of males is adaptive (submitted)

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Summary

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Introduction

The Glanville fritillary metapopulation - a model system in population biology

The Glanville fritillary metapopulation in the Åland Islands exhibits classic metapopulation structure and dynamics (Hanski 1999; Hanski et al. 1995), and it has become a widely recognized model system for understanding the rules of persistence of species living in fragmented landscapes, and for population biology in general (Ehrlich and Hanski 2004). The metapopulation occupies a large network of about 4,000 discrete small meadows within an area of 50 by 70 km, out of which about 500 meadows are occupied by a butterfly population at a time (Hanski 1999; Nieminen et al. 2004). The meadows are clustered into "semi-independent patch networks" that vary in the number of patches, patch sizes and connectivities. Annual surveys are conducted in the autumn, when all habitat patches are surveyed for the presence or absence of local populations and the number of larval groups is counted. The butterfly has gregarious larvae that spin a conspicuous winter nest, a feature that greatly facilitates annual surveys (Nieminen et al. 2004). A colonization event is recorded when one or more larval groups are found in a habitat patch that was unoccupied in the previous autumn. Similarly, an extinction event is recorded when no larval group is found in a habitat patch that was occupied in the previous autumn. Though the larval groups are quite conspicuous and extra care is taken to record the presence or absence of populations as carefully as possible, Nieminen et al. (2004) have estimated that the probability of missing an existing local population is from 0.10 to 0.15.

The metapopulation is characterized by a very high turnover rate of populations, close to 50%. Local populations are on average very small (4.3 family groups on average of mostly full-sib larvae), and new populations are frequently established by a single mated female (Hanski 1999; Hanski et al. 1995).

In Åland, adults fly in June to early July. Females are believed to usually mate only once in their lifetime in the field (Kuussaari 1998), although they were often found to regularly mate with two or even three males in an outdoor cage with relatively high population densities (Hanski et al. 2006). Females lay eggs in clusters of 100-200 on their host plants *Plantago lanceolata* or *Veronica spicata*, and the larvae hatch after two to three weeks. Because females only oviposit on the host plants, ovipositions can be easily monitored in experimental setups. Larvae overwinter in a winter nest that they spin at the end of August. Larvae start feeding again in the spring, and they pupate in May. There is a pronounced pattern of protandry, with males hatching on average 2-3 days earlier than females.

An important cause of mortality in natural populations of the Glanville fritillary is overwinter mortality: 20% of entire larval groups die during the winter. Overwinter mortality is known to be dependent on larval group size, small groups of less than 20 larvae having a very small chance of surviving (Kuussaari 1998; Nieminen et al. 2001).

The long-term empirical study of the Glanville fritillary has stimulated new modelling approaches in metapopulation theory by providing abundant, high-quality data to test model assumptions and predictions; and the study has thereby facilitated the development of metapopulation theory to be applicable to real metapopulations (Ehrlich and

Hanski 2004). The long-term project has also helped integration of ecological, genetic and evolutionary processes. Because ecological phenomena are variable in space and time, meaningful integration of ecological and genetic research on natural populations can only be achieved in model systems for which there is large-scale and long-term demographic information available. For instance, the extensive database on population sizes and other ecological factors in several hundreds local populations for many years allowed Saccheri et al. (1998) to control for the influence of ecological factors on extinction risk and to show that inbreeding depression increases the risk of extinction of small local populations. This demonstration would not have been possible if the research had not been conducted in the model system context. Another example of integration of ecological and evolutionary studies is the finding that extinction-colonization dynamics play a role in the evolution of host plant use (Hanski and Heino 2003). Research on the Glanville fritillary has also contributed much empirical information and stimulated novel modelling work on the evolution of migration rate in metapopulations. One key result is that colonizations select for increased migration rate in metapopulations with fast turnover rate (Hanski et al. 2002).

Although the Glanville fritillary system is very well known in many respects, several processes that are crucial for understanding the genetics of metapopulations are still unknown. For instance, concerning the colonization of empty habitat patches, it is known that females are able to lay several egg clutches. The number and relatedness of females colonizing new populations is therefore unknown when there is more than one egg clutch. Additionally, although it is assumed that females mate in their natal population prior to dispersal, this has not empirically been investigated. If only one or two females establish new populations, it is more likely that the populations will suffer from inbreeding depression. Inbreeding depression has been shown to occur in the laboratory for this species (Haikola et al. 2001), but never in natural populations. And although a correlation between low heterozygosity of genetic markers and high extinction risk has been established (Saccheri et al. 1998), the exact mechanism leading from inbreeding depression to population extinction has not been elucidated. Moreover, although females are assumed to generally mate only once, a large proportion of females has been observed to mate up to three times in a large outdoor cage experiment that was conducted in nearly natural conditions (Hanski et al. 2006). This large population cage experiment also provides information on reproductive success of individuals (Hanski et al. 2006), and thereby allows investigating questions related to the evolution of life-history traits and various behavioural strategies in greater detail than previously.

The aims of this thesis work were to elucidate some of the behavioural, population, and genetic processes that have remained poorly studied and which may greatly influence metapopulation dynamics. More specifically, I investigated patterns of colonizations in terms of number and relatedness of founders, and studied inbreeding depression resulting from population establishment by only one female for populations of different age. Additionally, I analyzed sperm precedence patterns in multiply mated females to address the evolution of polyandry in a metapopulation context. I also used that information to determine the reproductive success of males and investigate how adaptive is protandry.

Genetic variation in metapopulations

Genetic variation is most easily perceived as adaptive variation in morphology, behaviour and physiology. Beyond that, neutral genetic variation is a measure of a population's evolutionary potential, the "raw material" for evolution that enables organisms to adapt to changing conditions (Hedrick and Miller 1992). Neutral genetic variation is often measured as heterozygosity of neutral markers or as the additive genetic variance that underlies characters such as life-history traits and morphology. The amount of genetic variation present in a population is shaped by selection, breeding structure, genetic drift, gene flow and mutation, all processes that depend on the effective population size. Factors that decrease the

effective population size include small census population size, variance in reproductive success, population bottlenecks, non-random mating, unequal sex ratios, variable population size and polygynous mating systems (Gaggiotti 2003; Wang and Caballero 1999). The smaller the effective population size, the more important genetic drift becomes, the change in allele frequency from one generation to the next caused by random sampling of alleles. The reduction in fitness caused by drift changing allele frequencies away from those favoured by selection is expressed as drift load (Crow 1993). Genetic drift and mating among close relatives due to restricted mating opportunities can lead to inbreeding depression, a reduction in fitness due to the increased expression of the genetic load, as well as reduced opportunities to express overdominance (Keller and Waller 2002; Mitton 1993). Inbreeding can adversely affect development, growth, survival, and fecundity (Charlesworth and Charlesworth 1987) and it has been extensively studied in domestic animals and in captive populations (Allendorf and Leary 1986; Crnokrak and Roff 1999; Falconer and Mackay 1996), and more recently also in the wild, mainly through intensive long-term studies with deep pedigree knowledge (Coltman et al. 1999; Coulson et al. 1998; Keller et al. 1994).

Frequency-dependent selection, spatially heterogeneous selection and overdominance all result in balancing selection and could act to maintain genetic variation (Whitlock 2004). In structured populations, reduced heterozygosity causes a reduction in the amount of variation maintained by overdominance and results in a more pronounced segregation load (Whitlock 2002). On the other hand, epistatic interactions between loci can cause different alleles to be favoured locally and maintain genetic diversity through heterogeneous selection (Whitlock and Barton 1997).

The effects of population structure on the maintenance of genetic diversity are not straightforward and depend on the variance in fitness and degree of genetic differentiation among local populations (Whitlock and Barton 1997). However, it is well established that recurrent local extinctions and recolonizations, by increasing the variance in reproductive success among local populations and thus decreasing the effective population size, cause a major reduction in the genetic diversity maintained within local populations and in the total metapopulation (Pannell and Charlesworth 1999; Whitlock and Barton 1997).

Population bottlenecks are common in metapopulations with frequent extinctions and recolonizations, especially when new populations are established by only few founders. As the maximum fraction of genetic variation lost during a bottleneck is a function of the population growth rate (Nei et al. 1975), populations that recover quickly after a bottleneck lose little genetic variation even if the population was reduced to a few individuals only. However, although such bottlenecks may not have a large effect on heterozygosity, they will have a strong impact on allelic diversity and thereby constitute a long term threat to population viability by reducing its evolutionary potential (Allendorf 1986). The loss of genetic variation has the effect of decreasing the additive genetic variance, whereas when there are gene interactions the differentiation of populations will tend to cause the additive genetic variance to increase. Thus, whether the additive genetic variance will increase or decrease after a bottleneck in structured populations depends on the relative magnitude of these two effects (Goodnight 2004).

Most new mutations are deleterious, and they are eliminated by selection in large populations, but in small populations they can become fixed if $S < 1/Ne$ (Maruyama 1970). With hard selection, population structure always increases the response to selection, especially for nearly recessive alleles that can be expressed more strongly in structured populations due to the excess homozygosity. With soft selection, however, individuals are competing locally for resources, and as the relatedness between competing individuals is increased with population structure, the response to selection can be lowered relative to a non-structured population (Whitlock 2002). Although selection is often more effective in structured populations,

this effect is usually counterbalanced by a strongly decreased effective population size, so that the rate of accumulation of deleterious alleles tends to be strongly increased in structured populations (Higgins and Lynch 2001; Whitlock 2003). Thereby, slightly deleterious alleles tend to accumulate over time, reducing individual fitness and contributing to an increasing mutation load. Also the probability of fixation of beneficial alleles tends to be much reduced with population structure, as a result of the reduced effective population size. It can however be increased for some loci, especially for nearly recessive alleles that can be expressed more strongly in structured populations because of increased homozygosity. Additionally, the time taken for fixation of new alleles is also affected by population structure (Whitlock 2003).

Migration will tend to decrease the population mean fitness and hence reduce the level of local adaptation because migrants are likely to be poorly adapted to local conditions. This will result in migration load, which becomes particularly important when there are substantial differences in the selection coefficients among populations, and can even set range limits of species (Kirkpatrick and Barton 1997; Mayr 1963). Migration load is lowered with lower migration rate, whereas drift load, segregation load and local drift load tend to increase with reduced migration rate. Whether population structure increases or decreases mean fitness on average depends on a large number of circumstances and on the specifics of the species (Whitlock 2004).

Methods: microsatellite markers

Due to advances in laboratory techniques and methods of data analysis, genetic approaches for addressing ecological questions are becoming increasingly efficient and powerful (Selkoe and Toonen 2006). Microsatellites are one of the most popular marker type for these studies, because they are numerous, highly variable, well distributed through the genome, consistently scorable and comparable, and easy to use (Schlötterer 2004). Microsatellites are tandem repeats of 1-6 nucleotides that are found at high frequency in the nuclear genomes of most taxa and typically vary in length between 5 and 40 repeats. In molecular genetic studies mostly dinucleotides, trinucleotides and tetranucleotides are used (Li et al. 2002). Microsatellite sequences mutate frequently by slippage and proofreading errors during DNA replication, resulting primarily in a change in the number of repeats (Eisen 1999). Because alleles differ in the number of repeats and thus differ in length, they can be distinguished by high resolution gel electrophoresis, a much more rapid and cost-efficient method than sequencing. Primers are designed to bind to the generally conserved flanking regions of a microsatellite locus for amplification with polymerase chain reaction (PCR). PCR-based technology allows using even degraded DNA for the amplification and makes non-invasive sampling possible, as DNA can be extracted from e.g. hair or faecal samples. Because of their high mutation rate, on average 10^{-4} mutations per locus per generation, microsatellites exhibit high levels of allelic diversity (Schlötterer 2000).

There are however several drawbacks to microsatellite markers. There are some taxa for which marker isolation is still fraught with considerable failure rate (Selkoe and Toonen 2006), such as some marine inverterbrate (Cruz et al. 2005; Hedgecock et al. 2004), lepidopterans (Zhang 2004) and birds (Primmer et al. 1997). In addition, the mutational mechanisms of microsatellites remain unclear. In the infinite allele model (IAM) every mutation event creates a new allele, whereas in the stepwise mutational model (SMM) that is specific to microsatellites, one or more repeat units is added or subtracted at a constant rate. Even though in the few model organisms studied microasatellites seem to follow mainly the SMM model (Eisen 1999; Ellegren 2004), metrics using the SMM tend to be highly sensitive to violations of that model, and thus metrics using the IAM seem to be more robust and reliable (Landry et al. 2002). Most analyses are however insensitive to mutational mechanism (Neigel 1997). Another drawback of microsatellites is that because of the high mutation rate, alleles that do not differ in length are not necessarily identical by descent, mainly because of back-

mutations and convergence. This can however be accounted for in analyses (Estoup and Cornuet 1999; Slatkin 1995), and appears to be problematic only when comparing very genealogically distant groups (Angers et al. 2000). Microsatellite markers should also be tested for gametic disequilibrium, selective neutrality and Mendelian inheritance (Selkoe and Toonen 2006). Violation of Mendelian inheritance is mainly due to null alleles (Selkoe and Toonen 2006).

Problems in the amplification and scoring of alleles present the main drawback of using microsatellites (Selkoe and Toonen 2006). Sources of error include misinterpreting an artefact peak as a true allele, incorrect interpretation of stutter pattern, and null alleles (Selkoe and Toonen 2006). Proper sample preservation is particularly important, as it can reduce substantially technical difficulties with amplification (Dawson et al. 1998). It is also important to always include a positive control (Delmotte et al. 2001). Error rate can be calculated by repeating marker amplification and scoring in a random subset (Hoffman and Amos 2005).

Null alleles are a very important source of error, particularly in certain taxa, like Lepidoptera (Meglecz and Solignac 1998). They can be detected as heterozygote deficit when testing for Hardy-Weinberg equilibrium, or if some individuals repeatedly fail to amplify at just one locus. Other possible causes of heterozygote deficit include inbreeding, population structure and the Wahlhund effect (Nielsen et al. 2003). Biological causes of heterozygote deficit should nevertheless affect all loci similarly, whereas null alleles affect only single loci. Statistical approaches to identify null alleles are also available (Van Oosterhout et al. 2004). Another way to detect null alleles is to examine patterns of inheritance in a pedigree (Paetkau and Strobeck 1995). "Larger allele dropout" is another way that alleles can be missed, when the longer allele in a heterozygote does not amplify as well as the shorter one, and is too faint to be detected in the scoring process (Wattier et al. 1998). Redesigning primers or adjusting PCR conditions can often ameliorate null allele problems (Callen et al. 1993).

The development of microsatellites markers for Lepidoptera has proven extremely difficult (Meglecz et al. 2004; Meglecz and Solignac 1998; Zhang 2004), apparently because microsatellite sequences with almost identical flanking regions exist in multiple copies in the genome of lepidopteran species (Meglecz et al. 2004; Zhang 2004). This is widely supported by the fact that from all published microsatellite studies in Lepidoptera no more than five polymorphic loci were isolated per genomic library in 80% of the cases (Zhang 2004). In the first chapter of my thesis, I describe the isolation and characterization of five microsatellite markers for the Glanville fritillary butterfly. All of them were characterized by very broad size ranges and high degree of polymorphism, which is typical for Lepidopera (Flanagan et al. 2002; Harper et al. 2000; Keyghobadi et al. 1999; Meglecz and Solignac 1998). Highly variable microsatellites have distinct advantages and drawbacks (Selkoe and Toonen 2006). Genotype scoring errors may occur due to increased large allele dropout (Buchan et al. 2005) and increased stutter (Hoffman and Amos 2005), and they have high rates of homoplasy that can introduce bias into allele frequency estimates, dampen estimates of Fst values, and lead to substantial inflation of gene flow estimates (Gaggiotti et al. 1999; Slatkin 1995). On the other hand, highly variable loci have increase power to distinguish close relatives for parentage (Queller et al. 1993). In the following chapters I have used these microsatellites for parentage identification when measuring the lifetime reproductive success of males, determining the sperm-precedence pattern in multiply-mated females, and estimating the number of females that established new local populations in the metapopulation. I also used microsatellites to estimate the relatedness of individuals.

Using microsatellite markers to estimate inbreeding has been heavily crtiticized (Pemberton 2004; Slate et al. 2004) based on a study comparing microsatellite-derived estimates of heterozygosity to the heterozygosity obtained from pedigrees in sheep (Slate et al. 2004). That study has not investigated other microsatellite-based measures, like internal relatedness, but suggests that such

measures might be promising alternatives, as they incorporate population allele frequencies (Slate et al. 2004). One reason why microsatellite derived estimates of heterozygosity seemed to perform so poorly in that study might have been the very low variance in expected inbreeding coefficient, which made it very difficult for marker-based estimates of heterozygosity to capture differences between individuals (Slate et al. 2004). Because microsatellites do not seem to be very suited to estimate inbreeding coefficients of individuals, I only used them to estimate the average inbreeding of local populations and the relatedness between individuals. In these roles microsatellites seemed to perform well, and the estimates were in line with what was expected based on the known demographic history of populations.

Results and discussion

Colonizations

The number and relatedness of individuals colonizing a new population are key parameters in determining the amount of genetic variation in local populations and in the entire metapopulation as well as the degree of differentiation among local populations (Pannell and Charlesworth 1999). Two extreme colonization models have been investigated in the theoretical literature: in the propagule pool model colonizers come from the same local population whereas in the migrant pool model colonizers represent a subsample from the entire metapopulation (Pannell and Charlesworth 1999; Slatkin 1977; Wade and McCauley 1988). Although the genetic consequences of different colonization models have been investigated theoretically, the colonization pattern has rarely been described in real metapopulations (Gaggiotti et al. 1999). The second chapter of my thesis aims at determining how many females establish new local populations, and whether females mate in their natal patch before dispersal. Additionally, in this study, which I did together with Otso Ovaskainen and Ilkka Hanski, the genetic results are compared with the results of an advanced dispersal model parameterized for the Glanville fritillary. The model can be used to assess the expected number of females colonizing new populations given the sizes and spatial locations of local populations in the previous generation.

The dispersal model captured well the general pattern of colonizations. The high mortality during the early life instars (Nieminen et al. 2004) is one reason why there were rarely several larval groups from the same female in newly-established populations in the empirical data. Larval mortality is negatively correlated with larval group size (Kuussaari 1998) and it should therefore be particularly high for later clutches, as these tend to be small (Wahlberg 1995). Because our sampling was done in the autumn, all larval groups that had died before that were missed. Another reason why females did not lay several egg clutches in the same local populations is that they might not stay long enough in one patch to do so. The interval between ovipositions is usually two days, but it can be up to two weeks if weather conditions are unfavourable (Hanski, pers. obs.).

The genetic results show that butterflies establishing new populations were more related to their mate than to other parent butterflies in that population, implying that females mate in their natal local population prior to dispersal. These results confirm observations that females usually mate shortly after eclosure (Boggs and Nieminen 2004). Half of the colonizations consisted of only one larval group, indicating that these populations were established by only one mated female. This situation is an extreme example of propagule pool model with only two related founders and should result in very low genetic diversity in the newly founded populations. These populations are also likely to suffer from inbreeding depression, a subject I have investigated in the third chapter of this thesis. On the other hand, when there were several larval groups, the genetic results show that they were mostly established by several unrelated females. Because the females have nevertheless mated in their natal patch, this situation represents an intermediate one between the propagule pool model and the migrant pool model, with several unrelated groups of two related founders (a female and her mate) establishing a new population. In subsequent generations, matings among the offspring of these unrelated pairs

should result in higher fitness because of heterosis, a subject addressed in the third chapter of this thesis.

Inbreeding and local extinction

The Glanville fritillary butterfly metapopulation in the Åland Islands remains to date one of the only two systems in which a correlation between inbreeding (measured as low heterozygosity at genetic markers) and population extinction risk has been convincingly demonstrated (Frankham 2005; Saccheri et al. 1998). Although there is ample evidence for inbreeding depression in this species in the laboratory (Haikola et al. 2001; Nieminen et al. 2001), the extent of inbreeding depression has not yet been quantified in natural populations. In the third chapter of this thesis, in a study I did with Sari Haikola, I chose to concentrate on newly-established populations that were founded by only one female. From the result of the previous chapter it is evident that this is a very common situation in the metapopulation, as about half of the new populations had been established by a single female. Moreover, the fact that females were found to mate in their natal patch prior to dispersal makes these populations particularly prone to suffer from inbreeding depression in the generations following population establishment. The demographic knowledge about population age and population size that is available for many years allowed us to compare inbreeding depression in the generation immediately following population establishment ("new populations") to that in population that had remained small and isolated for several generations after establishment ("old populations"). Because in small populations all individuals might suffer of inbreeding depression due to genetic drift, the only way to measure the deleterious effects of inbreeding on fitness is by crossing individuals between populations and observing the fitness of their progeny (Hedrick and Kalinowski 2000; Keller and Waller 2002). A metapopulation context is ideal for this kind of study, because crossing individuals from different populations corresponds to what happens naturally when a migrant mates with residents (Ebert et al. 2002; Glemin et al. 2003; Haag et al. 2002; Keller and Waller 2002; Richards 2000).

Our study design consisted of conducting matings within and among new and old populations, and to measure offspring fitness as egg-hatching rate and larval survival to diapause. Additionally, inbreeding levels in new and old populations were estimated as average population heterozygosity at genetic markers, and pairwise relatedness between the two parental individuals was also computed. The metric I used was internal relatedness, a measure that takes into account allele frequencies in the population and that has been shown to be a much better estimate of inbreeding than previously used metrics (Amos et al. 2001). Populations that had been old and isolated for several years had strongly reduced heterozygosity levels compared to newly-founded populations, indicating rapid loss of genetic variation in these populations. Offspring fitness was strongly reduced in matings within old population, with a decrease in both egg-hatching rate and larval survival to diapause. Most importantly, as the reduction in egg-hatching rate and larval survival cumulatively contribute to reduced larval group size, these effects are further amplified by increased larval-group dependent overwinter mortality. Parental relatedness was found to be an important factor in determining offspring fitness, which declined sharply with increasing parental relatedness. This study provides an important link between the results of Saccheri et al (1998) of an increase in extinction risk in populations with low marker heterozygosity and laboratory results describing inbreeding depression in this species (Haikola et al. 2001). Our results show that inbreeding depression is regularly occurring in natural populations following establishment by only one mated female, especially if the population remains small for several generations. The combination of lowered egg-hatching rate and low larval survival amplified by elevated overwinter mortality is very likely to be the mechanism leading to population extinction in inbred populations in the wild. These results underline the importance of interactions between genetic and ecological factors in determining population fitness and extinction risk. Interestingly, most of the long-term studies of inbreeding depression in the wild report a similar interaction between unfavourable ecological conditions and the strength of

inbreeding depression (Coltman et al. 1999; Keller et al. 1994).

Life history evolution in metapopulations

The number of studies on life history evolution in the metapopulation context is surprisingly small (Ronce and Olivieri 2004). The field is largely dominated by theory, with very little empirical work, and it is dealing almost exclusively with questions related to the evolution of dispersal (Ronce and Olivieri 2004). The two last chapters of my thesis deal with the evolution of life history traits. The data come from a large outdoor cage experiment that was conducted in summer 2003, in which matings and ovipositions were recorded intensively, and the offspring were reared until diapause (Hanski et al. 2006). Such data are exceptionally well suited to the study of natural selection, because it allows determining the reproductive success of all individuals. The fourth chapter of my thesis deals with the evolution of multiple mating in females. Although multiple mating has been assumed to be rare in the Glanville fritillary butterfly, a large proportion of females were found to mate multiply, with up to three males, in the population cage. I used microsatellite markers to determine the sperm precedence pattern, and I found a very strong last-male sperm precedence. The data was used to study multiple matings in females, and also to determine the reproductive success of males in order to study protandry.

Both protandry (the earlier emergence of males than females) and polyandry (multiple mating in females) are common life-history phenomenon in insects (Morbey and Ydenberg 2001; Thornhill and Alcock 1983). Protandry was first described by Darwin in 1871, who postulated that protandry could have evolved by natural selection for maximizing the reproductive success of males (Darwin 1871). In addition to adaptive explanations of protandry, alternative 'incidental' hypotheses have been postulated, whereby protandry would arise as a 'by-product' of selection acting on females, but not on males, for increased body size. No selection would be expected on the timing of male emergence, and males emerging at different times should not differ in mating success (Baughman 1991). In addition, under this hypothesis, there should be a trade-off between developmental time and adult size (Nylin et al. 1993). These questions have mainly been addressed with theoretical models, and empirical data to support any of these hypotheses are scarce and controversial (Del Castillo and Nunez-Farfan 1999; Maklakov et al. 2004; Nylin et al. 1993; Wedell 1992), mainly due to the difficulties involved in estimating mating success in insects. My results demonstrate that incidental hypotheses to explain protandry are unlikely, as there was no correlation between adult size and development time in females or males. When estimating the strength of natural selection with these data, I could show that protandry is adaptive in males, although the strength and shape of natural selection is likely to vary in space and time. These results contradict predictions of evolutionary stable strategy models and support more deterministic models developed to explain the evolution of protandry. Nevertheless, the optimal level of protandry is likely to be influence by population density. At very low population densities pronounced protandry might be disadvantageous, as it would decrease the phenological overlap between potential mates. Also the metapopulation structure might affect the optimal level of protandry. Males that emerge late will be alive later in the breeding season, and will therefore be more likely to mate with an immigrant female and produce high-quality outbred offspring. Although females are expected to have already mated before dispersal, a significant proportion of females was found to mate multiply in the outdoor cage experiment. It would be interesting to compare levels of protandry in metapopulations and in more continuous populations at different population densities.

The last chapter of my thesis deals with the evolution of multiple mating in females. For males, reproductive success is expected to increase linearly with the number of mates, but the advantages of multiple mating for females are less clear (Yasui 1997). Mating is costly to females, because of time and energy costs, and often because of increased risk of predation, injury or infection (Blanckenhorn et al. 2002; Chapman et

al. 1995). Multiple mating by females has been explained in terms of direct benefits, particularly in species in which males provide females with a nutrient-rich ejaculate (Arnqvist and Nilsson 2000), and in terms of indirect genetic benefits (Fedorka and Mousseau 2002; Jennions and Petrie 2000; Kozielska et al. 2004; Newcomer et al. 1999). Indirect genetic benefits include both the inheritance of "good genes" by the offspring (Andersson 1994; Johnstone 1995; Wedell and Tregenza 1999), the avoidance of genetic incompatibility (Foerster et al. 2003; Zeh and Zeh 1996), and the increase in offspring genetic diversity (Watson 1991; Yasui 1998). In many species of Lepidoptera, the ejaculate transferred by the male to the female during mating functions as a nuptial gift which increases fecundity, egg size or longevity in multiply mated females (Boggs 1990; Wiklund et al. 1993). In a review of 122 experimental insect studies, (Arnqvist and Nilsson 2000) found that polyandry could be explained by direct benefits alone, even in species without nuptial gift. On the other hand, in their review of polyandry and fecundity in Lepidoptera, (Torres-Vila et al. 2004) found that re-mating had no detectable effects on fecundity in descriptive studies of monandrous species. Similarly, there is no evidence that polyandry leads to direct benefits in Drosophila melanogaster (Brown et al. 2004).

When pre-copulatory cues allow females to identify the genetic quality of potential mates, they can potentially engage in additional matings with genetically superior (e.g. (Hasselquist et al. 1996; Kempenaers et al. 1997; Pitcher et al. 2003) or more compatible males (Garner and Schmidt 2003; Masters et al. 2003). When females cannot reliably identify the genetic quality of males, they may rely on post-copulatory cues to increase the bias in fertilization by using sperm that will confer their offspring with the highest genetic benefits (Jennions and Petrie 2000). Males of higher genetic quality are more likely to produce high-quality ejaculate and have a higher share of paternity in sperm competition (Arnqvist 1989; Parker 1990). Post-copulatory paternity biasing for greater compatibility has also been observed (Clark et al. 1999; Evans and Marshall 2005), for example through preferential destruction of genetically more similar sperm (Bishop 1996). In addition, there is evidence that post-copulatory paternity biasing increases offspring viability (Konior et al. 2001). As a result of post-copulatory inbreeding avoidance, offspring survival increased with the number of mates both in the European adder (Madsen et al. 1992) and in the European sand lizard (Olsson et al. 1994). In a laboratory experiment with the yellow dung fly, superior sperm competitors sired higher-quality offspring (Hosken and Stockley 2003).

Finally, genetic bet-hedging (Gillespie 1973; Gillespie 1974; Gillespie 1975; Gillespie 1977; Hopper 1999; Seger and Brockman 1987) is another mechanism that could explain polyandry, especially when females mate indiscriminately (Fox and Rauter 2003; Yasui 1998; Yasui 2001). Bet-hedging is expected to reduce variance in fitness among individuals within one generation and to increase the geometric mean fitness of polyandrous females relative to that of monandrous females. By mating with several males, females can avoid having all their offspring fathered by a low-quality or an incompatible male (Fox and Rauter 2003; Jennions and Petrie 2000; Yasui 1998; Yasui 2001).

One reason why bet-hedging can be important is the metapopulation structure of the Glanville fritillary in the Åland Islands (Hanski 1999; Nieminen et al. 2004). When local populations can be so small that a single mated female can establish an entire new local population (Hanski 1999; Hanski et al. 1995), ensuring that at least some offspring are viable through variance reduction is a valid argument in favour of bet-hedging (Yasui 1998). This can be achieved either by mating multiply before dispersal, or alternatively mating in the new patch if males are encountered there. Re-mating after dispersal would provide the added benefit of mating with an unrelated male, which is likely to increase offspring fitness. The benefits of bet-hedging could be accentuated by the fact that the groups of gregarious larvae have to exceed a threshold size before they are likely to survive throughout the larval stage (Kuussaari 1998), which makes it particularly important to bet-hedge in order to

ensure that at least one larval group will be large enough to survive the winter.

In the last chapter of my thesis, in a study I did together with Hanna Kokko, we derive predictions of the bet-hedging hypothesis for the Glanville fritillary. The egg-laying and survival patterns are in line with the predictions we derive, supporting the hypothesis that multiple mating in the Glanville fritillary presents a rare case of within-generation bet-hedging. Because this study was based on the data from the outdoor cage experiment in which butterflies were unrelated, any incompatibility effects due to inbreeding depression are likely to be underestimated. Therefore, both the egg-laying pattern of polyandrous females and the resulting reduced variance in their life-time reproductive success suggest that within-generation bet-hedging is an unusually likely candidate for explaining the occurrence of polyandry as a successful evolutionary strategy in a metapopulation context.

Conclusions

The results of my thesis bring new understanding to the consequences of extinction-colonization dynamics on genetic and evolutionary processes in metapopulations. I focused primarily on colonizations (II and III), because in the Glanville fritillary metapopulation in the Åland Islands colonizations (and local extinctions) are very frequent and the system presents a prime example of colonizations by only few females (II). The results show that population establishment by only one mated female may lead to rapid inbreeding depression, which combined with elevated overwinter mortality due to reduced size of larval groups may ultimately lead to population extinction (III, Figure 1). These adverse effects of low genetic diversity are amplified even further by the fact that females usually mate in their natal population before dispersal (II, Figure 1). On the other hand, population establishment by several females is expected to increase greatly the genetic diversity of the newly-established populations, especially as the founder females are usually unrelated (II, Figure 1). In the next generation, mating among these unrelated groups should lead to high offspring fitness (III, Figure 1). Migration among local populations may additionally rescue small inbred populations by introducing new genetic variation (III, Figure 1).

Multiple mating in the Glanville fritillary butterfly seems to be much more common than previously assumed, as a large proportion of females was found to mate with two or three males in a large outdoor experiment (Hanski et al. 2006). Multiple mating in the natal population could alleviate the adverse effects associated with population establishment through a bet-hedging mechanism, which would allow the least related male (or the best male in some other regard) to sire more offspring (V). If females disperse to an existing population, re-mating after dispersal would harbour the added benefit of increased offspring fitness resulting from low parental relatedness (III). Therefore, multiple mating is likely to be maintained in the metapopulation context as a successful evolutionary strategy (Figure 1), in spite of the costs associated with it. Inbreeding depression associated with population establishment is also likely to have an influence on the evolution of other important life-history traits. One obvious trait is the evolution of dispersal. A metapopulation structure with frequent colonizations resulting in small inbred populations would favour highly dispersive individuals, because dispersers into such populations would enjoy strongly increased reproductive success (III). Other important life-history traits, like protandry, are also likely to be influenced by the metapopulation structure (IV, Figure 1). This demonstrates the importance of integrating genetic and ecological factors. Inbreeding depression and larval-group size dependent mortality due to various environmental factors combine to play a key role both in determining the extinction risk of small populations and in influencing the evolution of multiple mating in females (III and V).

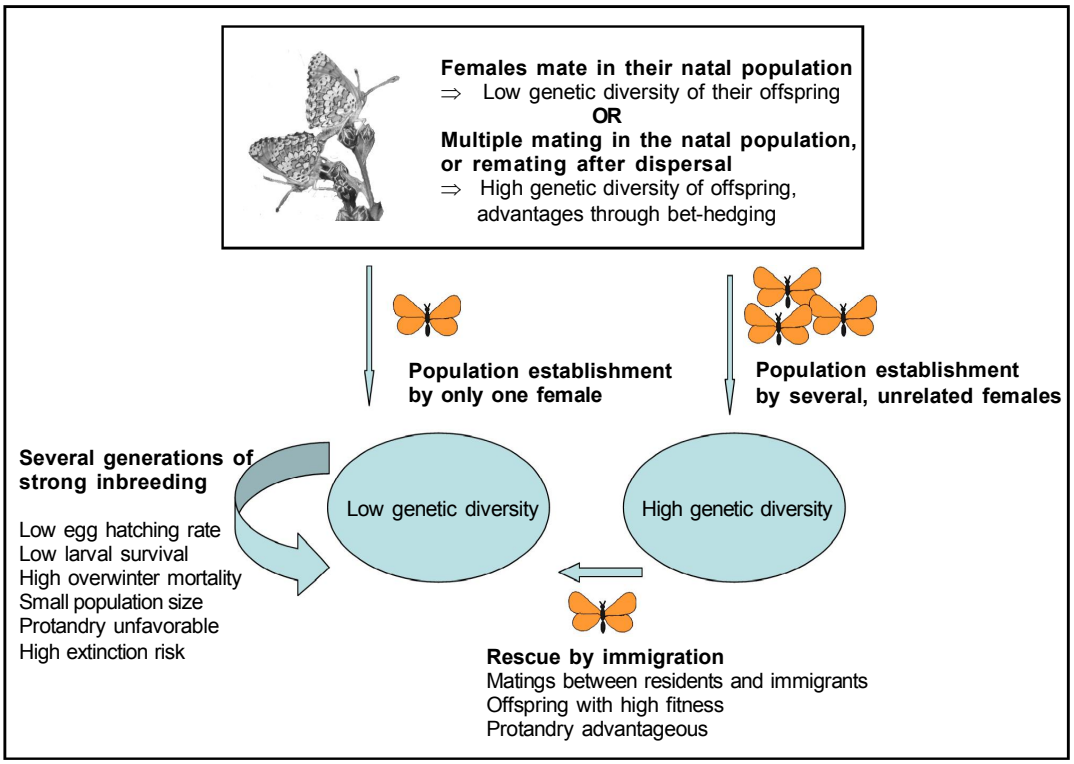

Figure 1. Effects of colonization pattern, female matings and immigration on the maintenance of genetic diversity in metapopulations

Box 1. Parentage analysis methods

modified after (Jones and Ardren 2003; Van de Casteele et al. 2001)

Genetic studies of parentage play a major role in the study of evolution and behavioural ecology. There are several methods available for reconstructing patterns of parentage (see below), and the appropriate technique for data analysis is dictated in large part by the type of samples that can be obtained from the study system and the available molecular markers. The best-case scenario is one in which large groups of offspring can be collected from known mated pairs (e.g. verifying parent offspring relationships to determine sperm precedence pattern in multiply mated females, **IV** and **V**). The situation becomes more difficult as the completeness of the sample diminishes (e.g. reconstruction of parental genotypes from groups of offspring, **II**).

Exclusion

- based on Mendelian rules of inheritance
- uses incompatibilities between parents and offspring to reject candidate parents
- best method if all or some of the potential parents are sampled and if no large sib- groups are available
- needs highly polymorphic markers and is very sensitive to mutations and genotyping errors

Categorical allocation and fractional allocation

- uses likelihood-based approaches to select the most likely parents from a pool of non-excluded candidate parents (Meagher and Thompson 1986)
- fractional allocation assigns some fraction, between 0 and 1, to all non-excluded parents, and likelihoods are calculated in the same way as in the categorical allocation method (Devlin et al. 1988)
- can be used in addition to exclusion methods if the markers are not polymorphic enough and there are still several non-excluded parents remaining
- allows for some amount of mutations and genotyping errors

Parental reconstruction

- uses multilocus genotypes of parents and offspring to reconstruct the genotype of unknown parents (Jones 2001)
- needs sampling of large groups of full- or half- sibs
- can be used even if candidate parents are not known
- needs highly polymorphic markers

Relatedness estimation

- neither of the parents is known, candidate parents cannot be sampled and no large sib-groups available, parentage cannot be reconstructed, relatedness techniques must be used
- several relatedness estimators have been proposed: a similarity index (Li et al. 1993), a regression based estimator (Queller and Goodnight 1989), and a correlation-based moment estimator (Lynch and Ritland 1999)
- typically large errors of inference (Lynch and Ritland 1999; Ritland 1996)

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